



<b>Title</b>	<b>Minimum Inhibitory Concentration (MIC) of antibacterial agents against cariogenic organisms</b>
<b>Author(s)</b>	<b>Botelho, MG; Samaranayake, LP</b>
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Variations in germ-tube formation amongst oral *Candida albicans* isolates from HIV-infected and uninfected individuals.  
NAIR RG\*, SAMARANAYAKE LP (Oral Biology Unit, Faculty of Dentistry, The University of Hong Kong, Hong Kong).

There is no data on germ-tube (GT) formation in *C. albicans* isolates associated with HIV-infection. Hence a total of 47 oral *C. albicans* isolates (29 from HIV-infected and 18 from uninfected individuals) was investigated using a previously described method using bovine serum and TC 199 medium as GT inducers. Briefly, 0.5 ml of *C. albicans* suspension ( $10^7$  cells/ml) was incubated at 37°C for 90 min with an equal volume of bovine serum. One hundred *Candida* cells were counted for GT formation using a haemocytometer, under 400x magnification. In addition, a select group of 6 isolates both from HIV-infected and uninfected individuals were pre-exposed to apo-lactoferrin (20 µg/ml) and lysozyme (20 and 50 µg/ml) followed by serum to evaluate their effect on GT induction. Amongst the media tested, serum produced more GT compared with TC 199 medium (mean 25.2 - 35.7% and 23.0 - 26.2%, respectively), irrespective of the source of *C. albicans*. When the GT forming ability of *C. albicans* isolates from HIV-infected and uninfected individuals was compared, a marginally high rate was observed amongst the former group ( $p > 0.05$ ). Whereas apo-lactoferrin (20 µg/ml) and lysozyme (20 µg/ml) had no effect, lysozyme at a concentration of 50 µg/ml suppressed the *C. albicans* GT formation. The foregoing indicates that serum is a better GT inducer compared with TC 199 medium, and isolates from HIV-infected individuals and those uninfected have no significant difference in GT forming ability.

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Minimum Inhibitory Concentration (MIC) of Antibacterial Agents Against Cariogenic Organisms  
MG Botelho\* & LP Samaranayake. Prince Phillip Dental Hospital, Hong Kong

While some research has investigated the MIC of antibacterial agents against bacteria associated with plaque, there has been little on the MICs of bacteria specifically isolated from carious lesions. A total of thirty one bacteria: streptococci (9), lactobacilli (10) and actinomyces (12); isolated from carious lesions were tested as to their minimum inhibitory concentration (MIC) against eight antibacterial mouthwash agents. The antibacterial agents were: chlorhexidine diacetate, chlorhexidine dihydrochloride, chlorhexidine gluconate, benzalkonium chloride, cetrimide, cetylpyridinium chloride, thymol and sodium hypochlorite. Using an accepted protocol, serial dilutions of the antibacterial agents were prepared in microtitre plates in concentrations from 64 µg/ml to 0.0625 µg/ml. An inoculum of the test organism equivalent to  $10 \times 10^5$  colony forming units per millilitre was dispensed into a microwell containing thioglycolate media and antibacterial agent. The tray was then incubated for 48 hours at 35°C. After this time, the lowest concentration at which no growth occurred was recorded to be the MIC. Thymol and sodium hypochlorite did not show any antibacterial activity at the concentrations tested. The MIC range for all the microorganisms was 8.0 to 0.125 µg/ml. There was no statistically significant difference between the MICs of the three chlorhexidines and the remaining 3 antibacterial agents. The results from this study indicate that while thymol and sodium hypochlorite have limited antimicrobial effect antibacterial agents commonly used in mouthwashes have similar MIC levels against cariogenic bacteria.

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Subgingival microflora and periodontal conditions in outpatients at Chulalongkorn University. H.NAPAWONGDEE J.KUWATANASUCHATI and R.MAHANONDA\* (Faculty of Dentistry, Chulalongkorn University, Thailand).

The aims of the present study were to evaluate the periodontal conditions using the CPITN and the new loss of attachment index of WHO and also to investigate the presence of *Porphyromonas gingivalis* in subgingival plaque of the outpatients at the Faculty of Dentistry, Chulalongkorn University. 288 patients were examined. By using the CPITN only, it was found that more than 55% of the patients under the age of 25 had gingivitis and the minority had periodontitis. In contrast, more than 60% of the older age group (>25 year old) had periodontitis. The use of the two indices together show that most of the younger age group with 4-5 mm probing depth did not have attachment loss. On the other hands, the patients above the age of 35 generally showed the measurement of attachment loss greater than probing depth. That might imply that the use of the CPITN in the younger age group seems to overestimate the prevalence and severity of periodontal destruction and vice versa in the older age group.

Thirty samples of subgingival plaque were taken from either upper first or second molars (one sample per one patient). Fifteen samples were from the periodontitis sites (CPITN=4), while the rest were from the healthy sites (CPITN=0). *P. gingivalis* was identified by culturing technique, Gram stain smear and the illumination test under UV light (365nm). *P. gingivalis* was detected in 86.67% of the sites with advanced periodontitis, while none was found in the healthy sites. The proportion of this organism to anaerobic bacteria in the diseased group ranged 0.16-18.67% with median of 4.22%, hence supporting the role of *P. gingivalis* in advanced periodontitis in the Thai population.

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Bacterial morphotype of supragingival plaque in Chinese subjects.  
K.Y. ZEE\*, L.P. SAMARANAYAKE (Department of Periodontology & Public Health and Oral Biology Unit, Faculty of Dentistry, The University of Hong Kong)

The aim of this study was to estimate the percentage distribution of different bacterial morphotypes of supragingival plaque in Chinese subjects by using the experimental gingivitis model. Seven healthy dental students were recruited from Prince Philip Dental Hospital. All the subjects were provided with once-a-week professional prophylaxis for 3 weeks in order to ensure gingival health. In the fourth week, after prophylaxis, the subjects began a 21-day period without any mechanical or chemical plaque control. Plaque along the buccal gingival margin of 16, 15, 14, 24, 25, 26 were removed on Day 1, 3, 5, 7, 14, 21 respectively with a sterile curette and dispersed onto a drop of distilled water placed on a microscopic slide. Gram stained smears were prepared for light microscopy. At 1000X magnification, the single cell seen was classified as Gram-positive or Gram-negative cocci, rod, filament, fusiform organism, spirilla and spirochaeta. A differential count of 200 organisms from 3-6 fields in the microscope were performed. The figure obtained for each group of microorganisms were then expressed as percentage of the total number of bacteria counted. Gingival crevicular fluid (GCF) flow were measured from 13-23 during the 21 days by using Periotron® machine. A total of 42 smears were obtained and examined. Results showed GCF flow increased during the 21-day period. Gm+ve microorganisms were the predominant type of bacteria throughout the 21 days (66-93%) while Gm+ve bacteria only constituted around (5-22%). The proportion of the shaped bacteria increased with the plaque age. This is in contrast to results from studies using the same methodology in Caucasian populations in which Gm+ve bacteria were the predominant organisms. Further studies in the Chinese population are needed to confirm or refute these findings.

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THE POTENTIAL EFFECT OF CATECHIN IN TEA LEAF ON STREPTOCOCCUS MUTANS  
R. R. OWEN\* (PEDODONTIC DEPT. FACULTY OF DENTISTRY PADJADJARAN UNIVERSITY, BANDUNG-INDONESIA)

The objective of the study was to determine the minimal concentration (MIC) of catechin in tea that could inhibit the growth of *Streptococcus mutans*. This bacteria is predominant in caries formation. Based on the MIC it could be decided a used dilution concentration (UDC) of catechin in tea.

The sample was scraping of caries lesion taken from children who visited Pedodontic Clinic - The Faculty of Dentistry Padjadjaran University. The extraction of catechin from green tea was done in The Tea Research Center Laboratory in Gembung-Indonesia.

A serial dilution method of NCCLS (1985) was used to determine the minimal inhibitory concentration of catechin to *Streptococcus mutans*.

The microscopic examination and the identification of the cultures indicated that *Streptococcus mutans* is detected predominantly in caries. The serial dilution test showed that the minimal inhibitory concentration (MIC) of catechin was 0.5 mg/ml.

The statistical analysis using Two Ways Analysis of Variance followed by Multiple Comparison showed that the number of *Streptococcus mutans* decreased significantly in 0.5 mg concentration of catechin with the optimal contact time of 3 to 5 minute. It is assumed that to be used as a mouth gargle a used dilution concentration (UDC) of catechin in tea is 2 mg/ml (4 x MIC). This study was supported by The SLDR - ADB, Loan No. 1013-IND.

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A Relation of Plasma Albumin Level to Calculus Deposit. Y. KENALI\*, F. ZAKARIA, SAMJAJA (Universitas Indonesia, Institut Pertanian Bogor, Puslitbang Gizi, Indonesia).

Previous studies have shown that a group of proline rich proteins in human saliva can act as inhibitors of calcium phosphate precipitation in dental calculus formation. Since some of native Indonesians still consume diet with a low protein level nowadays, and the fact that many Indonesians have high score of calculus index, the purpose of this preliminary study was to investigate the correlation between plasma albumin level (as a body protein index) and calculus deposit on the tooth surface. 34 people aged 22-50 years, in Bogor, Indonesia, were subjected in this study. Plasma albumin level was measured using spectrophotometer. Calculus index was used to assess calculus deposit. The result showed that there was significant negative correlation between the plasma albumin level and calculus index ( $r = -0.43$ ,  $P = 0.01$ ). It is concluded that the low plasma albumin level is one of the possible reasons which promote the deposit of calculus on the tooth surface.

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Strength analysis of fixation of sagittal split osteotomy. B. ANUCUL\*, P.D. WAITE, J. E. LEMONS and C. VLACHOS (Mahidol University, BKK, Thailand and UAB, Alabama, USA)

Six fresh frozen human mandible were used as three dimensional testing specimens. Bilateral sagittal split osteotomies were performed and 5-mm. advancements were made. The specimens were divided into plate-fixed group and screw-fixed group. All specimens were subjected to repeated compressive loads of 22.27N by using Instron machine. Parameters of strength were analyzed by elastic deformation, stiffness ratio, permanent deformation and breaking load. Data analysis were done by means of two-sided matched pair t-test. The mean elastic deformation under 22.27N was  $0.36 \pm 0.4$  mm in plate group and  $0.24 \pm 0.24$  mm in screw group which were not statistically different ( $0.60 < p < 0.80$ ). There was statistical difference between mean stiffness ratio of the two group ( $0.40 < p < 0.60$ ), while the plate group was  $232.58 \pm 153.24$  N/mm, the screw group was  $342.91 \pm 195.89$  N/mm. The mean load for permanent deformation were statistically different between two groups ( $p < 0.001$ ). The plate group was  $16.43 \pm 16.39$  N, whereas the screw group was  $34 \pm 5.79$  N. The mean breaking load of the plate group was  $23.5 \pm 5.59$  N while the screw group was  $63.33 \pm 20.38$  N. It was significantly three times higher than that of the plate group ( $0.01 < p < 0.05$ ). It is concluded that the bicortical screw fixation can resist higher load than monocortical plate fixation and it takes more imposed load to cause fixation failure in the bicortical screw system than the monocortical plate system.

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Biochemical Differences Between Endochondral Bone and Intramembranous Bone Matrix.  
A. TWITTY\*, A.M. RABIE and D. SHUM\* (Department of Children's Dentistry and Orthodontics, Department of Biochemistry, The University of Hong Kong.)

In a qualitative study, we reported that composite intramembranous bone induced more new bone than composite endochondral bone. In order to elucidate the factors behind the enhanced osteogenic activity of intramembranous bone, biochemical analysis of the extracellular matrices of both types of bone was carried out specifically for the presence of an angiogenic inhibitor. Endochondral bone (femur) and intramembranous bone (mandibles and parietal bone) were harvested from 3-4 month old New Zealand white rabbits. The bone was cleaned, defatted, dried and pulverized (particle size: 60-25mm) before being demineralized in 0.5M HCl. Protein was extracted dissociatively using 4M GuHCl and ultrafiltration was used to give a 10-100kDa molecular weight range. Heparin Sepharose Chromatography further separated the proteins before they were run on 15% SDS polyacrylamide gels under reducing conditions. Preliminary studies have shown that there is a difference in banding pattern between the two bone types with results showing the presence of a 28-29kDa protein in the heparin bound fraction of endochondral bone proteins and an apparent absence from the equivalent intramembranous bone fraction. Further investigations are being carried out to determine the nature of this protein in order to establish whether or not it is an angiogenic inhibitor. This study was supported by RCG Grant # 372/251/6425. University of Hong Kong.